AN EVALUATION OF MORPHOLOGICAL AND CYTOLOGICAL DATA SETS FOR THE PHYLOGENY OF HOMOSCLEROPHORIDA (PORIFERA: DEMOSPONGIAE)

GUILHERME MURICY

Muricy, G. 1999 06 30: An evaluation of morphological and cytological data sets for the phylogeny of Homosclerophorida (Porifera: Demospongiae). *Memoirs of the Queensland Museum* **44**: 399-409. Brisbane. ISSN 0079-8835.

Congruence and resolution of two independent data sets (morphology and cytology) were compared in a phylogenetic analysis of ten Mediterranean species representing four genera of Homosclerophorida (Demospongiae). Two aspiculate genera (*Oscarella*, *Pseudocorticium*) and two with a siliceous skeleton (*Plakina*, *Corticium*) were studied to assess the validity of the traditional classification recognizing two families Plakinidae and Oscarellidae, respectively with and without a skeleton, with *Discodermia polydiscus* (Tetractinomorpha) used as an outgroup. Cytological data showed highest consistency but poor resolution, support, and average performance, probably due to low number of informative characters. It was the only data set that supported the monophyly of Oscarellidae. Morphological data had better congruence and support than cytological data, and indicated the non-monophyly of Oscarellidae. Combined analyses yielded a 'total evidence' tree topologically similar to that of morphological data, although with greater bootstrap support (average 64.7% per branch). The combined data set should be chosen for classification of Homosclerophorida because it includes all evidence available and showed the best general performance with the indices measured. Therefore, a classification including *Oscarella* and *Pseudocorticium* within Plakinidae is supported over the alternative, less parsimonious option which recognizes Oscarellidae as monophyletic. ❒ *Porifera, Homosclerophorida, phylogeny, morphology, cytology, W Mediterranean.*

Guilherme Muricy (e-mail: muricy@acd.ufrj.br), Departamento de Invertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, s/nº., São Cristóvão, 20940-040 Rio de Janeiro, RJ, Brazil; 18 May 1999.

Over the last few decades several new, non-morphological characters have been proposed for sponge taxonomy (e.g., embryological, cytological, biochemical and molecular data), in an effort to overcome long standing problems in traditional classification based solely on morphology. However, the relative merits of different types of characters have rarely been evaluated in a phylogenetic framework (but see Hajdu & Van Soest, 1996). The present study compares the phylogenetic information content of morphological and cytological data sets in the resolution of a particular phylogenetic problem in Porifera, viz. the monophyly of Oscarellidae Lendenfeld, 1887.

Homoscleromorpha Lévi, 1973, with its single order Homosclerophorida Dendy, 1905, is widely accepted as a monophyletic group (e.g., Bergquist, 1978; Hartman, 1982; Van Soest, 1987; Diaz & Van Soest, 1994). Its synapomorphic traits include viviparous cinctoblastula larvae, a basement membrane lining the pinacoderm and choanoderm, and both exo- and endopinacocytes being flagellated (Boury-Esnault et al., 1984, 1995; Diaz & Van Soest, 1994). Its outgroup relationships are still debated; despite the fact that a relationship to Calcarea, Calcaronea, has been postulated based on shared tetractinal symmetry of spicules and possession of amphiblastula larvae (Van Soest, 1984; Grothe & Reitner, 1988), it is more generally accepted that these are homoplastic traits and Homosclerophorida is more closely related to Demospongiae, Tetractinomorpha, due to the shared presence of siliceous tetractinal calthrops spicules (Diaz & Van Soest, 1994). Homosclerophorida is usually divided into two families, Plakinidae Schulze, 1880 with five genera bearing tetractinal spicules and derivatives (*Corticium* Schmidt, 1862, *Plakina* Schulze, 1880, *Plakinastrella* Schulze, 1880, *Plakortis* Schulze, 1880, and *Placinolopha* Topsent, 1897; see Diaz & Van Soest, 1994), and the monotypical Oscarellidae with genus *Oscarella* Vosmaer, 1884 without a skeleton (Lévi, 1973; Bergquist, 1978; Hartman, 1982). The recently described genus *Pseudocorticium*

TABLE 1. Species of Homosclerophorida studied from Marseille, France. *Discodermia polydiscus* is an outgroup (Tetractinomorpha: Lithistida).

Species	Author	Site of Collection	Depth (m)	Specimens examined
Pseudocorticium jarrei	Boury-Esnault et al., 1995	Jarre Cave	15-20	25
Corticium candelabrum	Schmidt, 1862	La Vesse, Jarre Is.	5-20	30
Oscarella lobularis	(Schmidt, 1862)	La Vesse	15-35	27
Oscarella tuberculata	(Schmidt, 1868)	La Vesse, Jarre Is., Gameau Cave	5-35	32
Oscarella microlobata	Muricy et al., 1996	Jarre and Gameau Caves	15-20	8
Oscarella viridis	Muricy et al., 1996	Jarre Cave	15-20	6
Plakina monolopha	Schulze, 1880	Grand Congloue	15	3
Plakina trilopha	Schulze, 1880	Jarre Cave	15-20	12
Plakina endoumensis	Muricy et al., 1998	Endoume Cave	2-5	8
Plakina jani	Muricy et al., 1998	Gameau Cave	15-20	15
Discodermia polydiscus	Du Bocage, 1869	Gameau Cave	15-20	10

Boury-Esnault et al., 1995, although aspiculate, is more similar to *Corticium* than to *Oscarella* in its biochemical characters (allozyme patterns; Solé-Cava et al., 1992, as *Corticium* sp.) and in several morphological characters (presence of a cortex, leuconoid aquiferous system with diplodal chambers, cartilaginous consistency, perforated surface; Boury-Esnault et al., 1995). This led to a proposal to abandon the taxon Oscarellidae and to merge all homoscleromorph genera into a single family Plakinidae (Solé-Cava et al., 1992; Diaz & Van Soest, 1994; Boury-Esnault et al., 1995). The problem at the level of character evolution is whether the absence of skeleton in *Oscarella* and *Pseudocorticium* is a convergence, a plesiomorphy, or a synapomorphy. Phylogenetic relationships among genera in Homosclerophorida remain unstudied, and therefore the validity of Oscarellidae, containing *Oscarella* and *Pseudocorticium* (both aspiculate), is questionable. This is currently a major phylogenetic problem in the classification of Homosclerophorida.

In this study, the consistency and resolution of two data sets (morphological and cytological) were compared in a phylogenetic analysis of ten Mediterranean species in four genera of Homosclerophorida (viz., *Oscarella*, *Plakina*, *Corticium* and *Pseudocorticium*). *Discodermia polydiscus* Du Bocage, 1869 (Tetractinomorpha: Lithistida: Theonellidae), was used as outgroup in all analyses. Although *Discodermia* is part of a problematic group, the lithisthid sponges (family Theonellidae Lendenfeld), its close relationships with the Astrophorid families Ancorinidae Schmidt and Geodiidae Gray have been demonstrated by analyses of both morphological data and DNA sequences (Kelly-Borges & Pomponi, 1994). The hypothesis that ectosomal discotrienes of *Discodermia* are homologous (although distantly related) to plakinid calthrops, both having derived from a common tetractinal ancestor spicule, is implicit in the choice of this particular outgroup. Cytological characters have been proposed as useful taxonomic tools at the species level, both in Homosclerophorida (Boury-Esnault et al., 1984, 1992, 1995; Muricy et al., 1996, 1999) and in other sponge groups (e.g. Simpson, 1968; Pomponi, 1976; Vacelet & Donadey, 1987; Boury-Esnault et al., 1994). The present study aimed to compare the congruence and resolution of phylogenetic estimates derived from morphological and cytological data sets at the genus and family levels in Homosclerophorida. The two individual and combined data sets were also compared as to their support for the monophyly of Oscarellidae. Alternative hypotheses on phylogenetic relationships between the four genera of Homosclerophorida studied are discussed.

MATERIALS AND METHODS

Samples of ten homosclerophorid species and one outgroup species (Table 1) were collected using SCUBA between 1984-1995 from caves and vertical walls at 10-30m depth along the coast of Provence, Western Mediterranean, France (5°20'W, 43°10'N). For the morphological study, samples were fixed in formalin or glutaraldehyde/ sodium cacodylate, dehydrated in an alcohol series and included in Araldite. Thick and semi-thin sections were stained with toluidine blue and studied under light microscopy (LM). Spicules were observed under LM or scanning electron microscopy

(SEM) after sputter-coating with gold-palladium. Complete descriptions of each species studied and their important morphological and cytological characters are given elsewhere (Schulze, 1880; Ridley & Dendy, 1887; Topsent, 1895; Boury-Esnault et al., 1984, 1992, 1995; Muricy et al., 1996, 1998, 1999). Vouchers were deposited at the sponge collections of the Muséum National d'Histoire Naturelle, Paris (MNHN) and the Museu Nacional of the Universidade Federal do Rio de Janeiro, Brazil (UFRJPOR). For the cytological study, specimens were fixed as described by Boury-Esnault et al. (1984): glutaraldehyde 2.5% in a mixture of 0.4M cacodylate buffer and sea water (4 vol.: 5 vol.; 1120mOsm) and postfixed in 2% osmium tetroxide in sea water. Thin sections, contrasted with uranyl acetate and lead citrate, were observed under TEM in a Hitachi Hu 600 microscope. Cytological data on *Discodermia polydiscus* are based on the author's unpublished observations and those of N. Boury-Esnault (pers. comm., 1993).

All parsimony analyses were carried out using PAUP 3.0 (Swofford, 1991). In all searches, the following options were used: algorithm = branch and bound; keep minimal trees only; addition sequence furthest; collapse zero-length branches; multistate taxa = polymorphisms; optimization = ACCTRAN; rooting by outgroup (=*Discodermia polydiscus*); all characters unordered, with equal weights. Dependence between characters was checked by searching the data matrix for characters with identical distribution of states among taxa (Appendices 1,2). Although some characters were excluded by this method (e.g. presence/absence of spicules and of an ectosomal skeleton), total similarity in distribution of several characters was considered fortuitous (i.e. not artifacts of character coding), and these traits were thus kept in the phylogenetic analyses (characters 7, 18; 13, 15, 32, 33, 34; 16, 17; 19, 20; 21, 22; 23, 24; 38, 40; 41, 42). Presence/ absence of spicules was excluded from the analyses because it was a key-character in the hypotheses tested, and to avoid redundancy with characters 19-26 (spicule types). Its evolution was traced *a posteriori* using MacClade 3.0 (Maddison & Maddison, 1992), as well as that of all other unambiguous character-state changes. One thousand minimal random trees (MRTs) were generated and their length distribution was compared to the most parsimonious trees obtained for the real data. The observed length of the most parsimonious trees never exceeded that of the shortest randomized trees, and therefore it was accepted that the original data differed significantly ($P<0.001$) from randomness in all data sets. The following information was recorded for each individual and combined data sets: number of characters, number of informative characters, number of most parsimonious trees (MPTs), length of MPTs, number of extra steps (e), consistency index (CI), homoplasy index (HI), rescaled consistency index (RC), difference in steps between MRTs and MPTs, majority-rule consensus tree, number of branches involved in polytomies, bootstrap support for each branch, and unambiguous character-state changes supporting each branch (Swofford, 1991). These parameters measure either the internal consistency (congruence) of each data matrix, the resolution of phylogenetic estimates, or the support for the phylogenies derived from the different data sets. Bootstrap support for branches in consensus trees (Felsenstein, 1985; Li & Zharkikh, 1994) was calculated by 100 replicated branch and bound searches, with the same options held constant as described above. The number of extra steps in each analysis was used to measure incongruence due to disparity within and between data sets, analogous to analysis of variance (Mikevich & Farris, 1981; Kluge, 1989). Individual and combined data sets were ranked according to their relative performances on average for all indices measured, with the exception of extra steps and rescaled CI, which were redundant with HI and CI, respectively.

RESULTS

Of 30 morphological characters, 24 (80%) were phylogenetically informative for parsimony analysis, which resulted in three MPTs 67 steps long, with a fully-resolved majority-rule consensus tree (Fig. 1). MPTs of morphological data set required 14 extra steps, with intermediate consistency (CI=0.79, RC=0.61), and relatively high homoplasy (HI=0.25; Table 2). The majority-rule consensus tree (Fig. 1) supported monophyly of both *Plakina* and *Oscarella* (bootstrap=82-89%), but suggested non-monophyly of Oscarellidae. The alternative clade [*Plakina* + *Oscarella*] is supported by relatively low bootstrap (49%), but also by seven synapomorphies: small lobes, surface wrinkled, soft consistency, sylleibid canal system, eurypylous choanocyte chambers, ectosome between 5-50mm thick, and proportion of mesohyl to chambers less than 1:1 (Fig. 1). *Corticium*

TABLE 2. Summary of information on Homosclerophorida congruence. Abbreviations: MPTs, most parsimonious trees; MRTs, minimal random trees; e, number of extra steps; CI, consistency index; HI, homoplasy index; RC, rescaled consistency index.

Information	Morphology	Cytology	Combined
Character numbers	1-30	31-47	1-47
No. of characters	30	17	47
No. of informative characters	24	9	33
No. of MPTs (length)	3 (67)	7 (25)	1 (95)
CI	0.79	0.84	0.78
RC	0.61	0.65	0.57
HI	0.25	0.16	0.25
e	14	4	21
No. of MRTs (length)	1 (80)	1 (28)	4 (115)
MRTs minus MPTs	13	3	20
No. of branches in polytomies	0	2	0
No. of unambiguous changes	25	4	32
Mean bootstrap per branch (%)	62.2	40.7	64.7

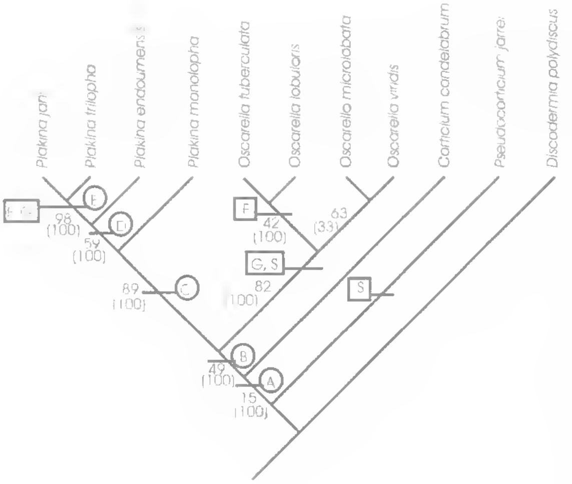

FIG. 1. Majority-rule consensus of 3 most parsimonious trees of the morphological data set. Numbers beside branches are bootstrap support for the branch, and the percentage of MPTs displaying the branch (within parenthesis). Synapomorphies (within circles) are indicated as 'character.state': A = 4.1; B = 5.1, 6.1, 10.4, 11.1, 12.1, 16.2, 17.2; C = 19.1, 20.1, 27.2; D = 23.1, 24.1, 25.1; E = 1.1, 6.0, 7.1, 10.3, 14.1, 16.1, 17.1, 18.1. Homoplasies (within rectangles) are: F = 5.2; G = 2.1, 4.2; S = absence of spicules. For the names of characters and states see Appendices 1-2.

candelabrum is placed as sister-group of the clade [*Plakina* + *Oscarella*], with *Pseudocorticium jarrei* as the most basal ingroup branch. Complete loss of spicules appeared convergently in *Oscarella* and *Pseudocorticium*. Bootstrap support per branch was intermediate among all analyses (average 62.2%). Morphological data were more informative for skeleton-bearing than for aspiculate species, as expected, since skeletal characters comprised 13 out of 30 morphological characters included in the analysis.

Parsimony analysis of cytological data yielded seven MPTs, of length 25 (4 extra steps). The cytological data set showed the smallest amount of homoplasy among all analyses (CI=0.84, RC=0.65, HI=0.16), but also the smallest number of informative characters (9 of 17; Table 2). All MPTs and their consensus (Fig. 2) supported the monophyly of Oscarellidae (with 54% of bootstrap support), with both genera *Oscarella* and *Plakina* indicated as paraphyletic, and *Corticium* placed in the most basal ingroup branch. Absence of spicules appeared as a synapomorphy for *Oscarella* and *Pseudocorticium*. Support to all resolved branches was generally low (average bootstrap 40.7%, 0-2 synapomorphies per branch). This was the only data set that suggested monophyly of Oscarellidae, including *Pseudocorticium jarrei* within the genus *Oscarella* as sister-species of *O. microlobata*. The clade [*Oscarella* + *Pseudocorticium*] is supported by synapomorphies 31.1 (triangular apopylar cells) and 44.0 (absence of sclerocytes). Cytological characters allowed better resolution and stronger support to the aspiculate homosclerophorid species than to the skeleton-bearing *Plakina* and *Corticium*.

The combined data set provided 33 informative characters (70.2%), which resulted in one MPT, 95 steps long (21 extra steps). As the combined data included all the homoplasy (incongruence) hypothesized within each data set and between data sets, consistency was slightly lower than that of any individual analyses (CI=0.78; RC=0.57; HI=0.25; Table 2). The single MPT (Fig. 3) supported the monophyly of both *Plakina* and *Oscarella*, with significant bootstrap (72-87%). It also supported the non-monophyly of the Oscarellidae, showing *Corticium* as the sister group of a clade [*Plakina* + *Oscarella*] which was supported by

TABLE 3. Rankings of data sets according to their performance in each index measured (data from Table 2) and on average. Abbreviations as for Table 2.

Index	Morphology	Cytology	Combined
No. of characters	2	3	1
No. of informative characters	2	3	1
No. of MPTs	2	3	1
CI	2	1	3
HI	2	1	2
MRTs minus MPTs	2	3	1
No. of branches in polytomies	1	3	1
No. of unambiguous changes	2	3	1
Mean bootstrap per branch (%)	2	3	1
Sum of rankings	17	23	12
Average ranking	2	3	1

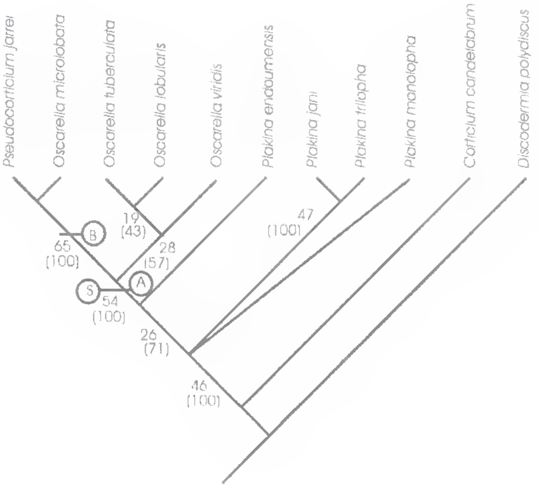

FIG. 2. Majority-rule consensus of 7 most parsimonious trees of the cytological data set. Key as for Figure 1. Synapomorphies (within circles) are indicated as 'character.state': A = 31.1, 44.0; B = 38.1, 40.1; S = absence of spicules. For the names of characters and states see Appendices 1-2.

66% of bootstrap and by 8 synapomorphies (small lobes, wrinkled surface, soft consistency, sylleibid canal system, eurypylous choanocyte chambers, ectosome between 5-50mm thick, proportion of mesohyl to chambers less than 1:1, and collencytes absent). Absence of spicules appeared homoplastic in *Oscarella* and *Pseudocorticium*, which was placed as the most basal ingroup branch.

Although the topologies suggested by the two data sets analyzed in this study were heterogeneous, their combination slightly increased the repeatability (as measured by the mean bootstrap support per branch) of the phylogenetic estimate when compared to the individual analyses. In general, the results of the combined data set were very similar in topology, resolution and support to those of the morphological data set, differing only in the relationships within *Oscarella*. The number of extra steps in each individual and combined analyses provided a direct measure of the incongruence within and between data sets. Of a total of 21 extra steps required by the most parsimonious trees for the combined data set, 86% (14+4) were due to incompatibility between characters within each data set, leaving 3 extra steps (14%) which reflect the incongruence between the two data sets (i_{mf}, Mikevich & Farris, 1981). Incongruence between sets was lower than within sets (16-20.1% in each of the individual analyses). A ranking of the average performance of individual and combined data sets (Table 3) was calculated based on their performance in each index measured (Table 2). The combined data set ranked best, followed closely by the morphological data set, and more distantly by the cytological data set.

DISCUSSION

Each of the two independent and combined data sets analyzed suggested a different phylogenetic reconstruction for Homosclerophorida, with varying degrees of resolution and support. The morphological data set showed better general performance when compared to the cytological data set (Tables 2, 3). Morphological data (particularly skeletal characters) are traditionally the major source of phylogenetic information for sponge taxonomists. Skeletal morphology provides valuable information in groups with high spicule diversity, such as Astrophorida and Poecilosclerida (e.g. Maldonado, 1993; Hajdu & Van Soest, 1996), but is of little use in skeleton-lacking species or in groups with low skeletal diversity such as Haplosclerida (e.g. Boury-Esnault et al., 1995; Muricy et al., 1996; Vacelet & Donadey, 1987; De Weerdt, 1989). Non-skeletal morphological characters (e.g., shape, surface, aquiferous system) comprised 17 out of 30 morphological characters studied, and 14 were informative for phylogenetic analysis.

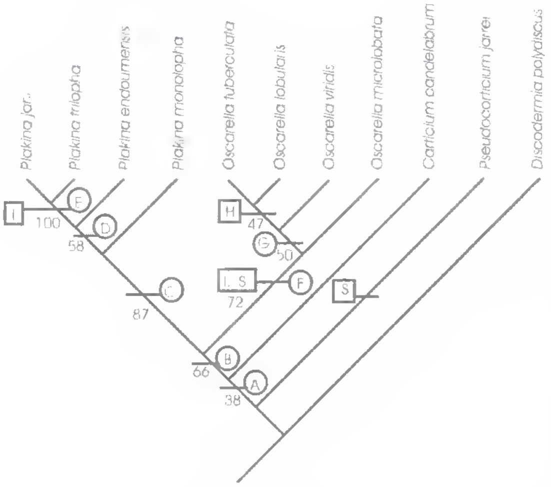

FIG. 3. Most parsimonious tree of the combined data set. Numbers beside branches are bootstrap support for each branch. Synapomorphies (within circles) are indicated as 'character.state': A = 4.1; B = 5.1, 6.1, 10.4, 11.1, 12.1, 16.2, 17.2, 45.0; C = 19.1, 20.1, 27.2; D = 23.1, 24.1, 25.1; E = 1.1, 5.2, 6.0, 7.1, 10.3, 14.1, 16.1, 17.1, 18.1, 47.1; F = 44.0; G = 46.0; H = 5.2. Homoplasies (within rectangles) are: I = 2.1, 4.2; S = absence of spicules. For the names of characters and states see Appendix 1.

Such non-skeletal characters, particularly the anatomy of the aquiferous system, can also be useful for the taxonomy of Homosclerophorida and other sponge taxa (e.g. Langenbruch, 1991; Bavestrello et al., 1995), and should be taken into account more often in sponge taxonomy.

The cytological data set showed poorest resolution, support, and general performance, although it had the highest consistency indices of all data sets. This was probably due to the low number of informative cytological characters. Cytology was more informative in *Oscarella* and *Pseudocorticium* than in *Plakina* and *Corticium*. This was expected since, in Homosclerophorida, the aspiculate species show greater diversity of cells with inclusions than the skeleton-bearing species (Boury-Esnault et al., 1992, 1995; Muricy et al., 1996, 1999). Cytological data also have proved useful for the systematics of other sponge groups such as Poecilosclerida, Haplosclerida and Hadromerida (e.g., Simpson, 1968; Pomponi, 1976; Boury-Esnault et al., 1994), and should be more frequently used in phylogenetic studies of all Porifera. However, phylogenetic interpretation of sponge cytology is presently difficult due to the scarcity of information available on cell function and chemistry, particularly of the so-called 'cells with inclusions' (Simpson, 1984). An increase in number of functional and cytochemical studies of sponge cells would greatly add phylogenetic information to cytological data.

How to choose the 'best' data set upon which classification should be based? One approach is to follow the principle of maximum evidence (e.g. Kluge, 1989), according to which the results of the combined analysis should be taken as a basis for classification of Homosclerophorida, since it was built with the maximum amount of independent evidence available. This approach has only been criticized when high between-set incongruence is found, because it may increase phylogenetic 'noise' and therefore reduce the accuracy of the combined analysis (Shaffer et al., 1991; Chippindale & Wiens, 1994; Wiens & Chippindale, 1994). Incongruence between data sets in Homosclerophorida (i_{mf}=0.14) is relatively low when compared to that found in other organisms (Shaffer et al., 1991; Olmstead & Sweere, 1994; Omland, 1994, and references therein). Therefore, it seems advisable in this case to base the classification on a combined data set. Another logical approach is to rank the data sets by their relative performances in each index of congruence, resolution and support recorded, and the data set with the best average performance would be chosen for classification. This approach is shown in Table 3, in which the sets were ranked according to data in Table 2. It shows that, on average, the combined data set performed better than any individual data sets. This approach also supports using the combined data set as a basis for classifying the Homosclerophorida.

The topology derived from the combined data set supported monophyly of *Oscarella* and *Plakina* and non-monophyly of Oscarellidae, with absence of spicules appearing as a homoplastic character in *Oscarella* and *Pseudocorticium*. Therefore, with the currently available data, a classification of Homosclerophorida with a single family Plakinidae Schulze (including Oscarellidae Lendenfeld and Corticiidae Vosmaer), as recently adopted by Solé-Cava et al. (1992), Diaz & Van Soest (1994), and Boury-Esnault et al. (1995), is preferred over the traditional classification with two families Plakinidae and Oscarellidae (Lévi, 1973; Bergquist, 1978; Hartman, 1982).

The analysis of independent data sets

obviously help to increase the amount of independent evidence upon which the phylogenetic hypotheses are based, and to reveal conflicts between data that can have strong influence in the choice of a classification. Furthermore, comparison of different data sets allows the determination of a 'phylogenetic confidence interval' for the phylogenetic hypothesis, which includes the topologies suggested by the individual and combined data sets (Bull et al., 1993; Huelsenbeck et al., 1994). Incongruence between data sets suggests that probably none of them has given the exact answer, and this should caution against changes in classification in face of new, conflicting data, before the congruence of the new evidence is more carefully evaluated. Therefore, the monophyly of Oscarellidae suggested by old classifications and by the cytological data set, although with relatively low support, cannot be completely ruled out at the current state of knowledge on biology of Homosclerophorida. It is kept as an alternative, less parsimonious hypothesis of phylogeny, together with the possibility of paraphyly of both *Oscarella* and *Plakina* suggested by cytological data, which should also be taken into account in studies on phylogeny of Homosclerophorida.

ACKNOWLEDGEMENTS

I am most grateful to M. Carpine (Institut Océanographique, Monaco), K. Smith (National Museum of Natural History, Washington), C. Valentine (Natural History Museum, London), R.W.M. van Soest (Zoological Museum, Amsterdam), and C. Lévi (Muséum National d'Histoire Naturelle, Paris) for the kind loans of specimens for taxonomy, and to C. Jalong for useful diving assistance. N. Boury-Esnault and J. Vacelet (Station Marine d'Endoume, Marseille) kindly assisted in morphology and cytology assessments, which were made at the Centre d' Océanologie de Marseille, and also provided useful discussions. Critical reading by E. Hajdu (Museu Nacional, Rio de Janeiro), S. Zea (Universidad Nacional de Colombia, Santa Marta) and J.N.A. Hooper (Queensland Museum, Brisbane) greatly improved the manuscript. The author has a fellowship from CNPq. This work was partly supported by CEE MAST-2CT91-004 program, CNPq, FAPERJ and FUJB/UFRJ.

LITERATURE CITED

BAVESTRELLO, G., BURLANDO, B. & SARÀ, M. 1995. Corrosion cast reconstruction of the three-dimensional architecture of Demosponge canal systems. Pp. 93-100. In Lanzavecchia, R., Valvassori, R. & Candia Carnevali, M.D. (eds) Body cavities: function and phylogeny. Selected Symposia and Monographs, U.Z.I. Vol. 8 (Mucchi: Modena).

BERGQUIST, P. R. 1978. Sponges. (Hutchinson & Co.: London).

BOCAGE, J.V.B. DU 1869. Éponges siliceuses nouvelles de Portugal et de l'Ile Saint-Iago (archipel du Cap Vert). Journal des Sciences Mathématiques, Physiques et Naturelles de l'Académie des Sciences de Lisbonne 2: 159-162.

BOURY-ESNAULT, N., DE VOS, L., DONADEY, C. & VACELET, J. 1984. Comparative study of the choanosome of Porifera: I. The Homoscleromorpha. Journal of Morphology 180: 3-17.

BOURY-ESNAULT, N., SOLÉ-CAVA, A.M. & THORPE, J.P. 1992. Genetic and cytological divergence between colour morphs of the Mediterranean sponge *Oscarella lobularis* (Schmidt) (Porifera, Demospongiae, Oscarellidae). Journal of Natural History 26: 271-284.

BOURY-ESNAULT, N., HAJDU, E., KLAUTAU, M., CUSTODIO, M. & BOROJEVIC, R. 1994. The value of cytological criteria in distinguishing sponges at the species level: example of the genus *Polymastia*. Canadian Journal of Zoology 72: 795-804.

BOURY-ESNAULT, N., MURICY, G., GALLISSIAN, M.F. & VACELET, J. 1995. Sponges without skeleton: a new Mediterranean genus of Homoscleromorpha (Porifera, Demospongiae). Ophelia 43: 25-43.

BULL, J.J., HUELSENBECK, J.P., CUNNINGHAM, C.W., SWOFFORD, D.L. & WADDELL, P.J. 1993. Partitioning and combining data in phylogenetic analysis. Systematic Biology 42: 384-397.

CHIPPINDALE, P.T. & WIENS, J.J. 1994. Weighting, partitioning, and combining characters in phylogenetic analysis. Systematic Biology 43: 278-287.

DENDY, A. 1905. Report on the sponges collected by Professor Herdman, at Ceylon, in 1902. Ceylon Pearl Oyster Fisheries, Supplementary Reports 18: 1-246.

DIAZ, M. C. & SOEST, R. W. M. VAN 1994. The Plakinidae: a systematic review. Pp. 93-110. In Soest, R. W. M. van, Kempen, Th.M.G. van & Braekman, J.C. (eds) Sponges in time and space. (Balkema: Rotterdam).

FELSENSTEIN, J. 1985. Confidence limits in phylogeny: an approach using bootstrap. Evolution 39: 783-791.

GROTHE, F. & REITNER, J. 1988. Discussion on the phylogenetic position of the Homoscleromorpha (Porifera). Berliner Geowissenschaften Abhandlungen, Reihe A, Geologischen und Palaeontologischen 100: 16-17.

HAJDU, E. & SOEST, R.W.M. VAN 1996. Choosing among Poriferan morphological characters within the cladistic paradigm. Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Biologie 66 (supplement): 81-88.

HARTMAN, W.D. 1982. Porifera. Pp. 640-666. In Parker, S.P. (ed.) Synopsis and classification of living organisms. Vol. 1. (McGraw-Hill: New York).

HUELSENBECK, J.P., SWOFFORD, D.L., CUNNINGHAM, C.W., BULL, J.J. & WADDELL, P.J. 1994. Is character weighting a panacea for the problem of data heterogeneity in phylogenetic analysis? Systematic Biology 43: 288-291.

KELLY-BORGES, M. & POMPONI, S.A. 1994. Phylogeny and classification of lithistid sponges (Porifera: Demospongiae): a preliminary assessment using ribosomal DNA sequence comparisons. Molecular Marine Biology and Biotechnology 3: 87-103.

KLUGE, A.G. 1989. A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). Systematic Zoology 38: 7-25.

LANGENBRUCH, P.-F. 1991. Histological indications of the phylogenesis of the Haplosclerida (Demospongiae, Porifera). Pp. 289-298. In Reitner, J. & Keupp, H. (eds) Fossil and Recent Sponges. (Springer-Verlag: Berlin, Heildelberg).

LENDENFELD, R. VON 1887. On the systematic position and classification of sponges. Proceedings of the Zoological Society London (1887): 558-662.

LÉVI, C. 1973. Systématique de la classe des Demospongiaria (démosponges). Pp. 577-631. In Brien, P., Lévi, C., Sarà, M., Tuzet, O. & Vacelet, J. (eds) Traité de Zoologie. Anatomie, Systématique, Biologie. (Sér. Ed. P-P. Grassé). Vol. 3. Spongiaires. (Masson et Cie.: Paris).

LI, W-H. & ZHARKIKH, A. 1994. What is the bootstrap technique? Systematic Biology 43: 424-430.

MADDISON, W.P. & MADDISON, D.R. 1992. MacClade: analysis of phylogeny and character evolution. Version 3.0. (Sinauer Associates: Sunderland, Mass.).

MALDONADO, M. 1993. The taxonomic significance of the short-shafted mesotriaene reviewed by parsimony analysis: validation of *Pachastrella ovisternata* Von Lendenfeld (Demospongiae: Astrophorida). Bijdragen tot de Dierkunde 63(3): 129-148.

MIKEVICH, M.F. & FARRIS, J.S. 1981. The implications of congruence in *Menidia*. Systematic Zoology 30: 351-370.

MURICY, G., BOURY-ESNAULT, N., BÉZAC, C. & VACELET, J. 1996. Cytological evidence for cryptic speciation in Mediterranean *Oscarella* (Porifera, Homoscleromorpha). Canadian Journal of Zoology 74: 881-896.

MURICY, G., BOURY-ESNAULT, N., BÉZAC, C. & VACELET, J. 1998. A taxonomic revision of the Mediterranean *Plakina* Schulze (Porifera, Demospongiae, Homoscleromorpha). Zoological Journal of the Linnean Society 124(2): 169-203.

MURICY, G., BOURY-ESNAULT, N., GALLISSIAN, M-F. & VACELET, J. 1999. Anatomy, cytology and symbiotic bacteria of four Mediterranean species of *Plakina* Schulze, 1880 (Porifera, Homosclerophorida). Journal of Natural History 33: 159-176.

OLMSTEAD, R.G. & SWEERE, J.A. 1994. Combining data in phylogenetic systematics : an empirical approach using three molecular data sets in the Solanaceae. Systematic Biology 43: 467-481.

OMLAND, K.E. 1994. Character congruence between a molecular and a morphological phylogeny for dabbling ducks (*Anas*). Systematic Biology 43: 369-386.

POMPONI, S.A. 1976. A cytological study of the Haliclonidae and the Callyspongiidae (Porifera, Demospongiae, Haplosclerida). Pp. 215-235. In Harrison, F.W. & Cowden, R.R. (eds) Aspects of Sponge Biology. (Academic Press: New York).

RIDLEY, S.O. & DENDY, A. 1887. Report on the Monaxonida collected by the H.M.S. 'Challenger' during the years 1873-1876. Reports on the Scientific Results of the Voyage of the H.M.S. 'Challenger' 1873-1876, Zoology 20: 1-275. (Her Majesty's Stationery Office: London, Edinburgh, Dublin).

SCHMIDT, O. 1862. Die Spongien des Adriatischen Meeres. (Wilhelm Engelmann: Leipzig).

1868. Die Spongien der Küste von Algier. Mit Nachträgen zu den Spongien der Adriatischen Meeres (drittes Supplement). (Wilhelm Engelmann: Leipzig).

SCHULZE, F. E. 1880. Untersuchungen über den Bau und die Entwicklung der Spongien. Die Plakiniden. Zeitschrift für wissenschaftliche Zoologie 34: 407-451.

SHAFFER, H.B., CLARK, J.M. & KRAUS. F. 1991. When molecules and morphology clash: a phylogenetic analysis of the north American ambystomatid salamanders (Caudata: Ambystomatidae). Systematic Zoology 40: 284-303.

SIMPSON, T.L. 1968. The structure and function of sponge cells: new criteria for the taxonomy of poecilosclerid sponges (Demospongiae). Bulletin of the Peaboby Museum of Natural History, Yale University 25: 1-141.

1984. The cell biology of sponges. (Springer Verlag: New York).

SOEST, R.W.M. VAN 1984. Deficient *Merlia normani* Kirkpatrick, 1908, from the Curaçao reefs, with a discussion on the phylogenetic interpretation of Demosponges. Bijdragen tot de Dierkunde 54(2): 211-219.

1987. Phylogenetic exercises with monophyletic groups of sponges. Pp. 227-241. In Vacelet, J. & Boury-Esnault, N. (eds) Taxonomy of Porifera from the N.E. Atlantic and Mediterranean Sea.

NATO ASI Series, ser. G, Vol. 13. (Springer-Verlag: Berlin).

SOLÉ-CAVA, A.M., BOURY-ESNAULT, N., VACELET, J. & THORPE, J.P. 1992. Biochemical genetic divergence and systematics in sponges of the genera *Corticium* and *Oscarella* (Demospongiae: Homoscleromorpha) in the Mediterranean Sea. Marine Biology 113: 299-304.

SWOFFORD, D.L. 1991. PAUP: Phylogenetic analysis using parsimony, Version 3.0. (Illinois Natural History Survey: Champaign, Ill.).

TOPSENT, E. 1895. Étude monographique des Spongiaires de France. II.- Carnosa. Archives de Zoologie Expérimentale et Génerale 3: 493-590.

1897. Spongiaires de la Baie d'Amboine. Revue Suisse de Zoologie 4: 421-487.

VACELET, J. & DONADEY, C. 1987. A new species of *Halisarca* (Porifera, Demospongiae) from the Caribbean, with remarks on the cytology and affinities of the genus. Pp. 5-12. In Jones, W.C. (ed.) European Contributions to the Taxonomy of Sponges. (Sherkin Island Marine Station Publications: Sherkin Island, Ireland).

VOSMAER, G.C.J. 1884. Porifera. Pp. 65-176. In Bronn, H.G. (ed) Die klassen und ordnung des Thier-reichs. (Winter'sche Verlagshandlung: Leipzig).

WEERDT, W.H. DE 1989. Phylogeny and vicariance biogeography of north-Atlantic Chalinidae (Haplosclerida, Demospongiae). Beaufortia 39(3): 55-88.

WIENS, J.J. & CHIPPINDALE, P.T. 1994. Combining and weighting characters and the prior agreement approach revisited. Systematic Biology 43: 564-556.

APPENDIX I. Morphological and cytological characters and character-states used in phylogenetic analysis. Uninformative characters are marked with an asterisk (*).

MORPHOLOGICAL CHARACTERS.

1. **Shape:** 0-spherical; 1-thin spreading; 2-lobate/spreading; 3-small circular; 4-cushion; 5-lobate/pending.
2. **Size** (surface cover in cm^2): 0-small (<10); 1-large (>10).
3. **Thickness** (mm): 0-thin (1-5); 1-medium (5-20); 2-thick (20-50).
4. **Fixation:** 0-firm; 1- by thin filaments; 2-loose but without filaments.
5. **Size of lobes** (mm): 0-absent; 1-small (1-5); 2-medium (5-20); 3-large (20-50).
6. **Surface**: 0-smooth; 1-wrinkled; 2-perforated.
7. **Arrangement of ostia**: 0-dispersed; 1-alveolar pattern.

8*. **Oscula**: 0-low; 1-with elevated rim.

9. **Colour**: 0-white/cream; 1-light brown; 2-yellow; 3-green; 4-variable.
10. **Consistency**: 0-rigid; 1-cartilaginous; 2-semi-cartilaginous; 3-firm but compressible; 4-soft; 5-fragile.
11. **Canal system**: 0-leuconoid; 1-sylleibid.

APPENDIX 1 (cont.). Morphological and cytological characters and character-states used in phylogenetic analysis. Uninformative characters are marked with an asterisk (*).

12. Choanocyte chambers type: 0-aphodal or diplodal; 1-eurypilous.

13*. Chamber diameter (µm): 0-small (10-30); 1-large (30-70).

14. Subdermal cavities: 0-absent; 1-present.

15*. Basal cavity: 0-absent, 1-present.

16. Ectosome thickness (µm): 0-greater than 100; 1-between 50 and 100; 2-between 5 and 50.

17. Proportion of mesohyl to chambers: 0-greater than 2; 1-between 1 and 2; 2- less than 1.

18. Spicule malformations: 0-absent; 1-present.

19. Diods: 0-absent; 1-present.

20. Triods: 0-absent; 1-present.

21. Smooth calthrops: 0-absent; 1-present.

22. Monolophose calthrops: 0-absent; 1-present.

23. Dilophose calthrops: 0-absent; 1-present.

24. Trilophose calthrops: 0-absent; 1-present.

25. Tetralophose calthrops: 0-absent; 1-present.

26*. Candelabra (heterolophose calthrops): 0-absent; 1-present.

27. Position of first ramification in lophose actines: 0-absent; 1-proximal; 2-medial.

28*. Second ramification in lophose actines: 0-absent; 1-distal.

29. Lophose actine ends: 0-absent; 1-simple; 2-with terminal spines.

30*. Desmata, oxea, and discotriaenes: 0-absent; 1-present.

CYTOLOGICAL CHARACTERS.

31. Apopylar cells: 0-absent; 1-triangular; 2-ovoid, with large osmiophilic inclusions.

32*. Flagellum in pinacocytes: 0-absent; 1-present.

33*. Pinacocyte shape: 0-'T-shaped'; 1-flat/ovoid.

34*. Basement membrane: 0-absent; 1-present.

35. Vacuolar cells type A: 0-absent; 1-present.

36*. Vacuolar cells type B: 0-absent; 1-normal, sparse; 2-turgid, in groups.

37. Vacuolar cells type C: 0-absent; 1-present.

38. Paracrystallin inclusions cells: 0-absent; 1-present.

39*. Homogeneous inclusions cells: 0-absent; 1-present.

40. Single inclusion cells: 0-absent; 1-present.

41*. Crescent cells: 0-absent; 1-present.

42*. Microgranular inclusions cells: 0-absent; 1-present.

43*. Spherulous/microgranular inclusions cells: 0-absent; 1-present.

44. Sclerocytes: 0-absent; 1-present.

45. Collencytes: 0-absent; 1-present.

46. Endosymbiont bacteria: 0-few, sparse; 1-diverse, abundant.

47. Distribution of endosymbiont bacteria in the mesohyl: 0-random/uniform; 1-patchy; 2-close to choanocytes; 3-far from choanocytes.

APPENDIX 2. Data matrix of the combined data set (characters 1-24). Names of characters and character-states are listed in Appendix 1.

Taxon	Character																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Pseudocorticium jarrei	5	1	1& 2	0	3	2	0	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Plakina jani	1	1	1	2	2	0	1	1	2	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Plakina trilopha	1	1	1	2	2	0	1	1	0	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Plakina endoumensis	3	0	0	1	1	1	0	1	0	4	1	1	1	0	1	2	2	0	1	1	1	1	1	1
Plakina monolopha	3	0	0	1	1	1	0	1	0	4	1	1	1	0	1	2	2	0	1	1	1	1	0	0
Oscarella tuberculata	2	1	1& 2	2	2	1	0	1	4	2	1	1	1	0	1	2	2	0	0	0	0	0	0	0
Oscarella lobularis	2	1	1& 2	2	2	0	0	1	4	4	1	1	1	0	1	2	2	0	0	0	0	0	0	0
Oscarella microlobata	2	1	1	2	1	1	0	1	1	4	1	1	1	0	1	2	2	0	0	0	0	0	0	0
Oscarella viridis	2	1	1	2	1	1	0	1	3	5	1	1	1	0	1	2	2	0	0	0	0	0	0	0
Corticium candelabrum	4	0	1	1	0	2	0	1	1	1	0	0	1	0	1	0	0	0	0	0	1	1	0	0
Discodermia polydiscus	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX 2 (continued). Data matrix of the combined data set (characters 25-47). Names of characters and character-states are listed in Appendix 1.

Taxon	Character																						
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
Pseudocorticium jarrei	0	0	0	0	0	0	1	1	1	1	0	0	1	1	1	1	0	0	0	0	1	1	3
Plakina jani	1	0	2	1	2	0	2	1	1	1	0	0	1	0	0	0	0	0	0	1	0	1	1
Plakina trilopha	1	0	2	0	1	0	2	1	1	1	0	0	0	0	0	0	0	0	1	0	1	1	1
Plakina endoumensis	1	0	1	0	2	0	2	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Plakina monolopha	0	0	2	0	1	0	2	1	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0
Oscarella tuberculata	0	0	0	0	0	0	1	1	1	1	0	2	0	0	0	0	0	0	0	0	0	0	0
Oscarella lobularis	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Oscarella microlobata	0	0	0	0	0	0	1	1	1	1	1	0	0	1	0	1	0	0	1	0	0	1	0
Oscarella viridis	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0
Corticium candelabrum	1	1	1	0	1	0	2	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	2
Discodermia polydiscus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0